ESCMID Study Group for Biofilms

Executive committee 2012: Chairman: Niels Høiby, Denmark (hoiby@hoibyniels.dk), Vice Chairman: Gianfranco Donelli, Italy, Secretary: Christine Imbert, France, Treasurer: Thomas Bjarnsholt, Denmark, Member: Antonio Oliver, Spain, Member: Johan van Eldere, Belgium, Coopted member (Eurobiofilms 2013): Tom Coenye, Belgium, Coopted member (Eurobiofilms 2015): Veronika Holà, (Czech Republic)

Bacterial biofilms are structured communities of bacterial cells enclosed in a self produced polymeric matrix, adherent to a surface. The majority of bacterial chronic infections are due to bacterial biofilms. Studies of biofilms have revealed differentiated, structured groups of cells with community properties. Understanding of the genetics and molecular basis may provide new therapeutic targets and means to control biofilm infections.

Biofilm structures

Biofilms constitute a protected mode of growth that allows survival in the hostile environment. The biofilm consists of microcolonies encapsulated by exopolysaccharide (EPS) produced by the bacteria (Figure 1- 4), but most of the biofilm is made of water channels which operate as a distribution system of nutrients and oxygen (Figure 5).

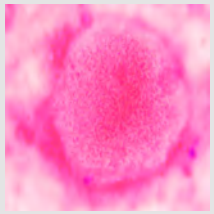


Figure 1
Gram-stained sputum from a 44 years old cystic fibrosis (CF) patient with mucoid and non-mucoid P. aeruginosa *since 1970. A detached alveolus with a* P. aeruginosa *biofilm is seen (x25 & x100) (Høiby, 2004).*

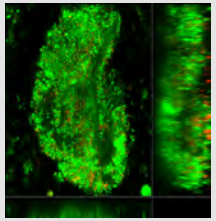


Figure 2
Confocal laser scanning microscopy (CSLM) image of Live/dead stained sputum sample from patient with CF (green cells are alive and red cells are dead) (J.A.J. Haagensen, 2005).

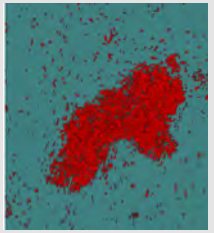


Figure 3
CSLM image of sputum sample from patient with CF after fluorescent in situ hybridization (FISH). A P. aeruginosa *specific probe (red) identifies* P. aeruginosa *colony directly in the sputum sample (J.A.J. Haagensen and L.Yang, 2005).*

Biofilms, inflammation and quorum-sensing

EPS, especially the polysaccharide alginate (mucoid phenotype) has an important role and function in CF. It acts as a scavenger of free oxygen radicals, prevents phagocytosis, and binds many antibiotics such as aminoglycosides (Figure 6).

P. aeruginosa controls the production and secretions of virulence factors by cell-to-cell communication signals in a process called quorum sensing (QS). The QS communication apparatus is composed of the *Las* and the *Rhl* systems. *P. aeruginosa* biofilms employs the QS signals to manipulate the host immune-response (Figure 7).

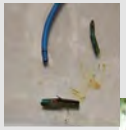


Figure 4A
Biliary stents with biofilm and amorphous material both on the inside and outside of the stent.

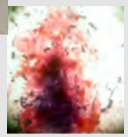


Figure 4B
Gram-stain (x1000) micro-scopic picture of the bacteria in the biofilm, Gram-negative rods and Gram-positive streptococci (Johansen et al, 2005).

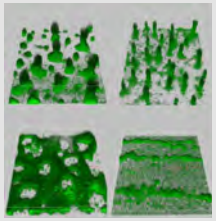


Figure 5
CLSM images of various structures of 7 days old flow-cell biofilms of Gfp tagged P. aeruginosa *from chronically infected CF patients (Lee et al., 2005).*

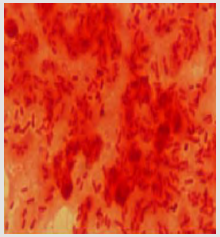


Figure 6
Gram-stained sputum from a CF patient. P. aeruginosa *biofilm surrounded by PMNs (Høiby, 2004).*

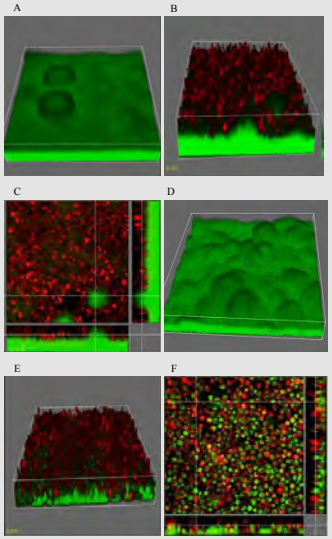


Figure 7
PMNs and non-mucoid P. aeruginosa *biofilm in vitro – (CLSM pictures)*
A) Wild-type PAO1 biofilm (gfp tagged). B) 2.5 h with PMNs stained with SYTO62 (PMN appear red fluorescent).C) Cross-section showing the wild-type biofilm with PMNs on top. D) ΔlasR-rhlR PAO1mutant. E) 3-D projection and F) Cross-section showing the ΔlasR-rhlR mutant fully penetrated by PMNs and the disappearance of much of the biomass (Bjarnsholt, T, Jensen, P.Ø. et al., Microbiology, 151,373-83, 2005).

Biofilms and antibiotics

Biofilm bacteria are generally more tolerant to antibiotic treatment than their planktonic bacteria counterpart. Antibiotic doses which kill suspended cells, for example, need to be increased as much as 1,000 x to kill biofilm cells (Figure 8). Biofilms evade antimicrobial challenges by multiple mechanisms. These have been grouped into three broad categories:
1) reduction of the antimicrobial concentration in the bulk fluid surrounding the biofilm (Figure 9).
2) failure of the antimicrobial agent to penetrate the biofilm; and
3) adoption of a resistant physiological state or phenotype by at least a fraction of the cells in the biofilm.

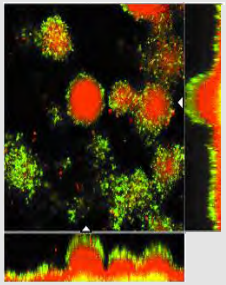


Figure 8
Biofilm protects the bacteria from the antibiotic treatment. Induction of β-lactamase in biofilm treated with ceftazidime (CLSM pictures).
PAO1 with PampC-gfp (ASV): 6 days old biofilm exposed to 100μg/ml ceftazidime (125 X MIC) for 4 h. Gfp expression indicates expression of AmpC β-lactamase in the biofilm. Detection level of the monitor: 10 μg/ml ceftazidime (Bagge et al., Antimicrob Agents Chemother. 2004; 48:1168–74).

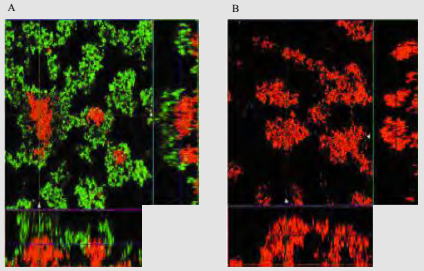


Figure 9
PAO1 with PampC-gfp (ASV): 6 days old biofilm exposed to 0.5 μg/ml imipenem (1/80 MIC) for 4 hours (A) and uninduced biofilm (B). Gfp expression indicates expression of AmpC β-lactamase in the biofilm. (Detection level of the monitor ≥0.1 μg/ml imipenem) (Bagge et al., Antimicrob Agents Chemother. 2004;48:1168–74).

ESCMID STUDY GROUP FOR BIOFILMS (ESGB) – founded April 2005 during 15th ECCMID in Copenhagen - will organize the following activities:

1) Propose an annual ESGB workshop to be held in conjunction with the annual ECCMID.
2) Suggest an annual biofilm symposium during the ordinary scientific programme of the annual ECCMID.
3) Organize an annual biofilm workshop between the annual ECCMIDs consisting of invited lectures and a poster session.
4) Establish a ESGB website with a ESGB Newsletter with information about the activities of the study group. This informations will also be communicated to all members of ESCMID in ESCMID NEWS.
5) During the annual workshops, educational activities including hands-on technical education will be planned and organized if technical possible.
6) Suggest guidelines for prophylaxis, diagnosis and therapy of biofilm infections.
7) An active cooperation with the ASM biofilm meetings will be proposed.

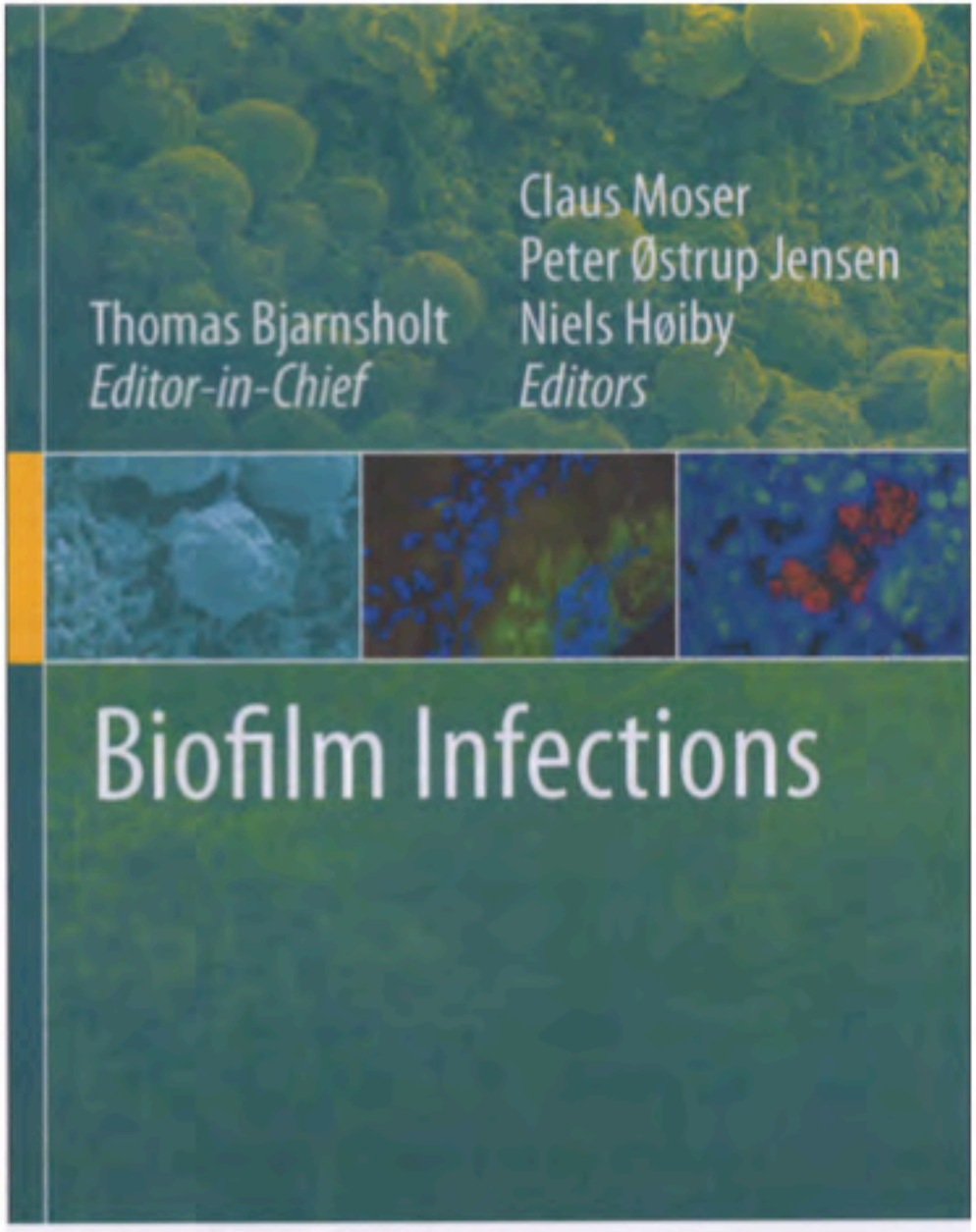
- **ESGB performance 2005-2011:**
- **ESBG 2005-ESCMID-ECCMID: Key-note lectures/2hr & 1hr symposia/educational workshops, 2006-11**
- **ASM-Biofilmconference Quebec 2007**
- **BAMBHAR/Mallorca 2007**
- **DTU, Lyngby, Technical Course 2009, 2010**
- **EUROBIOFILMS 2009 Rome**
- **Biofilms in nosocomial fungal infections Paris 2011**
- **Book: Biofilm Infections, Springer, 2010**
- **EUROBIOFILMS 2011 Copenhagen**
- **On-line course in biofilm : www.biofilmcourse.ku.dk, University of Copenhagen 2011**

- **ESGB future plans:**
- **ESBG 2005-ESCMID-ECCMID: Key-note lectures/2hr & 1hr symposia/educational workshops,2012 etc.**
- **DTU, Lyngby, Technical Course 2012**
- **Consensus conference: Diagnostic and treatment of biofilm infections 2012 together with ESGIAI**
- **EUROBIOFILMS 2013, 2015 etc.**

ESGB 2011 report

2011 has been a successful year for our Study Group. A new book 'Biofilm Infections' (pringer) was published by T. Bjarnsholt, C. Moser, P.Ø. Jensen & N. Høiby (eds) with 17 chapters by 52 international authors on all aspects of medical biofilms and biofilm techniques. Then followed a highly successful ESCMID postgraduate education course "Biofilm in nosocomial fungal infections" organized at Institut Pasteur on January 31st to February 1st 2011 which were attended by approximately 130 participants. The chair organizers, Christine Imbert and Anne Beauvais have to be congratulated for their very successful efforts. In July 2011 we had the Eurobiofilms 2011 in Copenhagen on July 5th to 8th 2011 which was the second European biofilm meeting which we have organized (the first one was in Rome 2009) and again the meeting was big success with more than 300 participants from 31 countries from 6 continents listening to 6 key note lectures, 13 symposia, 66 oral presentations (40 also presented as posters) and studying additional164 posters. The day before the congress we organized 4 workshops with 58 participants. A special issue of FEMSIM is being published with articles from Eurobiofilms 2011. Thomas Bjarnsholt, teasurer of ESGB also organized a new on-line course in Biofilms in the autumn 2011 through University of Copenhagen(www.biofilmcourse.dk) which can also be found on WWW.escmid.org/ESGB.
We have also in 2011 decided that the Eurobiofilms 2013 will take place in September 2013 in Gent, Belgium, organized by Tom Coenye, Johan van Eldere and Patrick van Dijck and the Eurobiofilms 2015 will take place in Brno, Czech Republic organized by Veronika Holá. Additionally we will organize the third ESGB technical course "Medical Biofilms Techniques" at DTU on August 27th to 30th 2012. We are also in the planning stage of organizing a consensus and guidelines meeting on diagnosis and treatment of medical biofilms together with two other study groups of ESCMID (Study Group of Nosocomial Infections and Study Group for Implant-Associated Infections).

Niels Høiby
On behalf of the board of ESGB



Springer November 2010

ESGB ESCMID STUDY GROUP FOR BIOFILMS
European Society of Clinical Microbiology and Infectious Diseases

We are repeating the success

Based on the great evaluation and success of the first course during the fall of 2011 we are now opening for applications for the 2012 online course on "Bacterial Biofilms and Their Role in Chronic Diseases". The course can be followed by both Master degree and PhD students, as well as professionals that live up to the admission criteria. For registration and more information, please visit:

www.biofilmcourse.ku.dk

Application deadline is 1 June 2012 (MSc- and prof.)
Application deadline is 6 August 2012 (PhD students)

Teachers at the course:

Director J. William Costerton
Centre for Genomic Sciences, Allegheny-Singer Research Institute, USA

Assoc Prof Marvin Whiteley
Section of Molecular Genetics and Microbiology, The University of Texas at Austin, USA

Prof Matt Parsek
Department of Microbiology, University of Washington, USA

Director, Phil Stewart
Chemical and Biological Engineering, Montana State University, USA

Prof, Niels Høiby, MD, Claus Ernst Moser and Dr, Peter Østrup Jensen
Department of Clinial Microbiology, Copenhagen University Hospital, Denmark

Luanne Hall-Stoodley
Southampton Wellcome Trust Clinical Research Facility and University of Southampton Respiratory BRU, Southampton General Hospital, UK

Assoc Prof, Thomas Bjarnsholt, Prof Michael Givskov and Assoc Prof Tim Tolker-Nielsen
Faculty of Health Sciences, University of Copenhagen, Denmark

Prof, Søren Molin
Department for Systems Biology, Technical University of Denmark

2nd FUNGAL BIOFILMS MEETING GLASGOW (UK), MAY 20th & 21st 2013

Local Organizers

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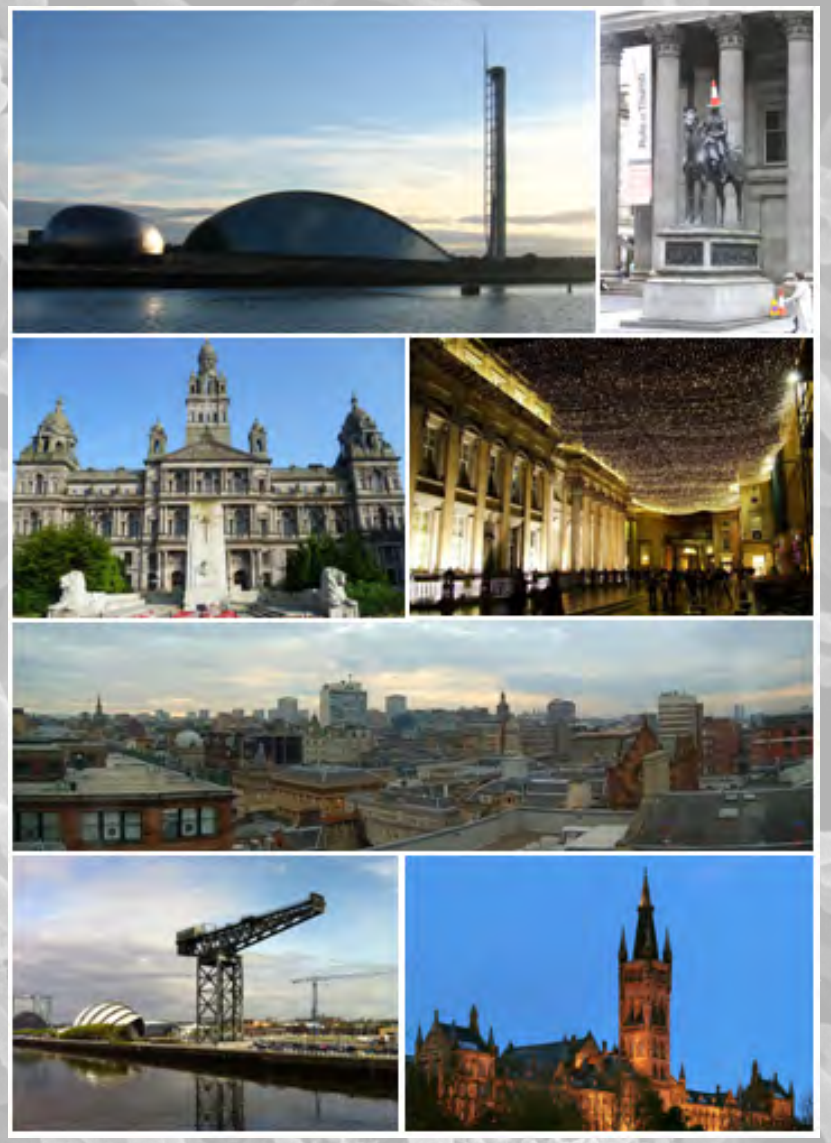


Organising Committee

Prof Craig Williams
Dr Gordon Ramage
Dr Anne Beauvais
Dr Christine Imbert
Prof Niels Hoiby

The Trades Hall of GLASGOW

http://www.tradeshallglasgow.co.uk/

Preliminary Sessions

Clinical implications of fungal biofilms
Pathogenesis and Inflammation
Polymicrobial biofilms
Biofilm technologies
Antifungal resistance
Molecular aspects of fungal biofilms

Prices (2 days, lunch, refreshments, reception)
Professional £150 (£100 per day) Student £50

ESGB
ESCMID STUDY GROUP FOR BIOFILMS

Confirmed Speakers

Dr David Andes (Madison, USA)
Dr Anne Beauvais (Paris, France)
Prof Geraldine Butler (Dublin, Ireland)
Dr Tom Coenye (Ghent, Belgium)
Prof Marcel Guttierez-Correa (Lima, Peru)
Dr Mariana Henriques (Braga, Portugal)
Dr Christine Imbert (Poitiers, France)
Prof Howard Jenkinson (Bristol, UK)
Prof Aaron Mitchell (Pittsburgh, USA)
Dr John Morrissey (Cork, Ireland)
Dr Carol Munro (Aberdeen, UK)
Dr Gordon Ramage (Glasgow, UK)
Dr Riina Rautemaa-Richardson (Manchester, UK)
Prof Jose Lopez Ribot (San Antonio, USA)
Prof Craig Williams (Glasgow, UK
Dr David Williams (Cardiff, UK)

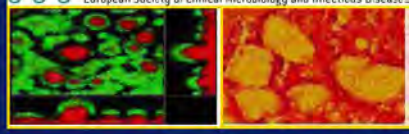
EUROBIOFILMS 2013
Third European Congress on Microbial Biofilms
Basic and Clinical Aspects

GHENT, BELGIUM, SEPTEMBER 9-12, 2013

www.eurobiofilms2013.ics.dk

ESGB ESCMID STUDY GROUP FOR BIOFILMS

European Society of Clinical Microbiology and Infectious Diseases



Organising Committee: Tom Coenye (president), Patrick Van Dijck (vice-president, basic), Johan Van Eldere (vice-president, clinical) and the ESGB Executive Committee

Scientific Advisory Board: Niels Hoiby, Gianfranco Donelli, Christine Imbert, Thomas Bjarnsholt, Craig Williams, Antonio Oliver, and Veronika Hola. More to be confirmed.

Conference Venue
Ghent University Aula, Voldersstraat, Ghent, Belgium

UNIVERSITEIT GENT

Pre-conference workshops: Pre-conference workshops will be organised on 9 September 2013. Topics of the workshops will include "Identification of biofilm-associated micro-organisms" and "Animal models for studying biofilms". More topics will be announced in the near future.

Main Sessions will take place from 9 September (evening) until 12 September (lunch time). Topics that will be addressed include mixed-species biofilms, microbial fuel cells, biofilm-related chronic wound infections, signalling in biofilms, clinical and basic aspects of fungal biofilms, mechanisms of biofilm resistance & tolerance, medical-device related biofilm infections, oral biofilms, biofilm heterogeneity & evolution, and microbial biofilm ecology.

Abstract Submission
You are kindly invited to submit abstracts describing research work that you wish to present at the Congress, either orally or as a poster. Deadline for abstracts for poster and oral sessions: March 15th, 2013. Guidelines for submission will be available on the congress website in the near future.

Registration & Hotel Accommodation
Information on the registration and hotel booking will be available on the conference website.

Congress Secretariat:
International Conference Services A/S
Copenhagen, Denmark,
Mail: eurobiofilms2013@ics.dk

Scientific Secretariat:
Laboratory of Pharmaceutical Microbiology
Ghent University, Gent, Belgium
Mail: eurobiofilms2013@ugent.be

Supported by European Society of Clinical Microbiology and Infectious Diseases (ESCMID) / www.escmid.org

ESCMID EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

UNIVERSITEIT GENT

www.eurobiofilms2011.ics.dk

hoiby@hoibyniels.dk

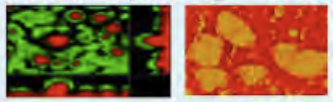
ESCMID Study Group for Biofilms

www.escmid.org**/esgb**

Website: http://www.esgb.eu

EUROBIOFILMS 2011
Second European Congress on Microbial Biofilms – Basic and Clinical Aspects

COPENHAGEN, DENMARK, JULY 6-8, 2011



Organising Committee: Niels Høiby (president), Søren Molin (vice president, basic), Gianfranco Donelli (vicepresident, clinical), Thomas Bjarnsholt (secretary) and the ESGB board

Scientific Advisory Board: Alejandra Bosch, Oana Ciofu, Gianfranco Donelli, Johan van Eldere, Alain Filloux, Hans-Curt Flemming, Michael Givskov, Christine Imbert, Peter Østrup Jensen, Helle Krogh Johansen, Staffan Kjelleberg, Per Klemm, Michael Kühl, Paolo Landini, Claus Moser, Per H. Nielsen, Bente Nyvad, Marco Oggioni, Antonio Oliver, Robert Palmer, Matt Parsek, Holger Rohde, Claus Sternberg, Philip Stewart, Paul Stoodley, Søren Sørensen, Kazuhiro Tateda, Tim Tolker-Nielsen, Paul Williams, Chen Yiqiang

Conference Venue
Panum Institute, University of Copenhagen, DK

Target Audience
This multi diciplinary conference target clinical microbiologists, infectious disease doctors, dentists, vetenarians, basic scientists working within the biofilm area and students on all levels.

Abstract Submission
You are kindly invited to submit abstracts describing research work that you wish to present at the Congress, either orally or as a poster. Abstracts must be submitted no later than 1 March 2011. Please consult the congress website for guidelines for submission www.eurobiofilms2011.ics.dk.

Registration & Hotel Accommodation
Information on the registration and hotel booking can be found on the website www.eurobiofilms2011.ics.dk where you can register online or download the registration and hotel form.

Registration Fee
The registration fee includes the scientific sessions, printed congress material, lunches and coffee breaks, but not travel and accommodation costs (see congress website www.eurobiofilms2011.ics.dk)

CME Accreditation
The organizer of the congress will apply for European CME accreditation

Precongress Workshops: Animal models of biofilms, Microfluidic systems and confocal microscopy, Molecular diagnostics of biofilm bacteria, New techniques for visualization of biofilms, Micro-sensors

Main Sessions: 'Diagnosis of biofilm infections', 'Regulation of biofilm development', 'Treatment of biofilm infections', 'Airway and wound biofilm infections and host response', 'Novel methodology for studies of biofilm structure and composition and omics', 'Dental biofilms', 'Diversification and evolution in biofilms', 'Biofilm-based medical device related infections and host response', 'Biofilm community ecology', 'Resistance and tolerance of biofilms to antibiotics and host response', 'Fungal biofilms', 'Social interactions in Biofilms' 6 key-note lectures, poster session.

Deadline for abstracts for poster and oral sessions: March 1st, 2011.

Scientific Secretariat:
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Mail: tbjarnsholt@sund.ku.dk

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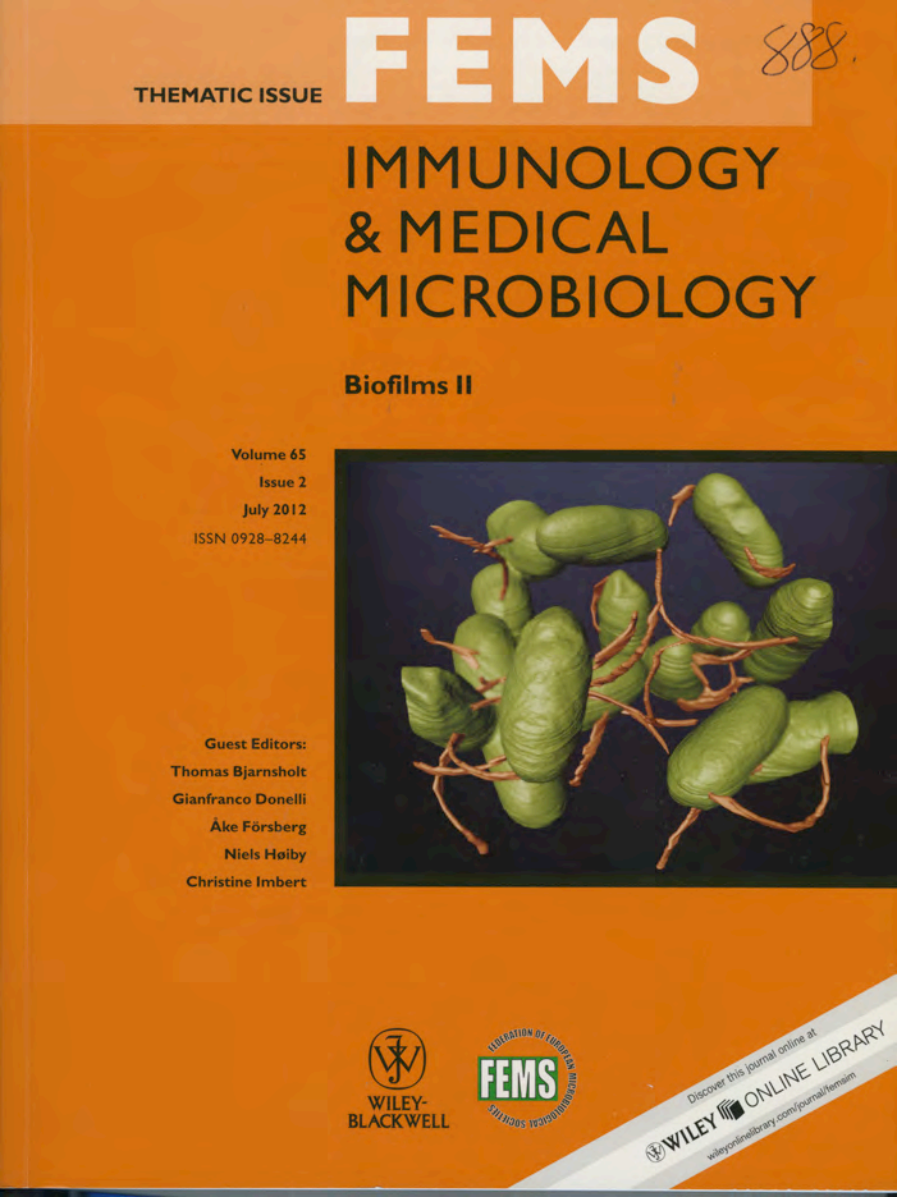

30 Biofilm Articles auhtored by speakers at EUROBIOFILMS 2011, Copenhagen, Denmark, July 2011. Dedicated to J.W. Costerton who died May 2012.

We would like to thank the West China School of Stomatology biofilm conference team and the *IJOS* editorial team for their hard work in preparation of this special conference and *IJOS* issue. We also wish to thank all sponsors for their generous support.

Keynote Speakers in the International Symposium of Microbial Biofilm
Chengdu China April 6th-8th, 2011

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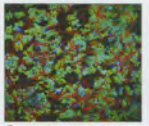
Contents

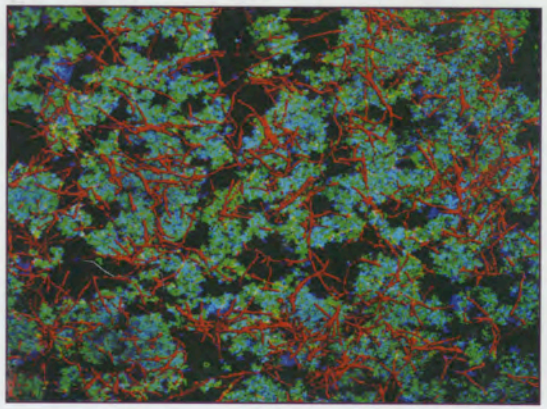


Cover: Representative confocal micrograph of a three-genera biofilm grown in a flow cell using saliva as the sole nutritional source for growth. See page 51 by Paul E. Kolenbrander for detail.

First Chinese international Biofilm Conference, Chengdu, PR China, April 2011

微生物生物膜与感染

Microbial Biofilm and Infection



主 编 周学东 施文元

人民卫生出版社

微生物生物膜与感染

Microbial Biofilm and Infection

名誉主编 闻玉梅 Bill Costerton Neils Hφiby

主 编 周学东 施文元

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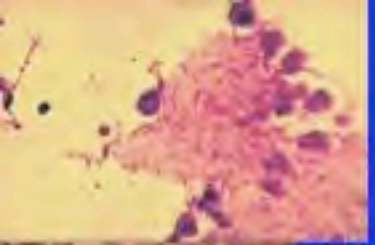
First Chinese textbook about Biofilms, published 2012



What is a biofilm?

- Appearance - how does it look
- Where is it located/persists: Surface: - body - outer/inner
  Surface: - artificial
  Inside body

Gradients

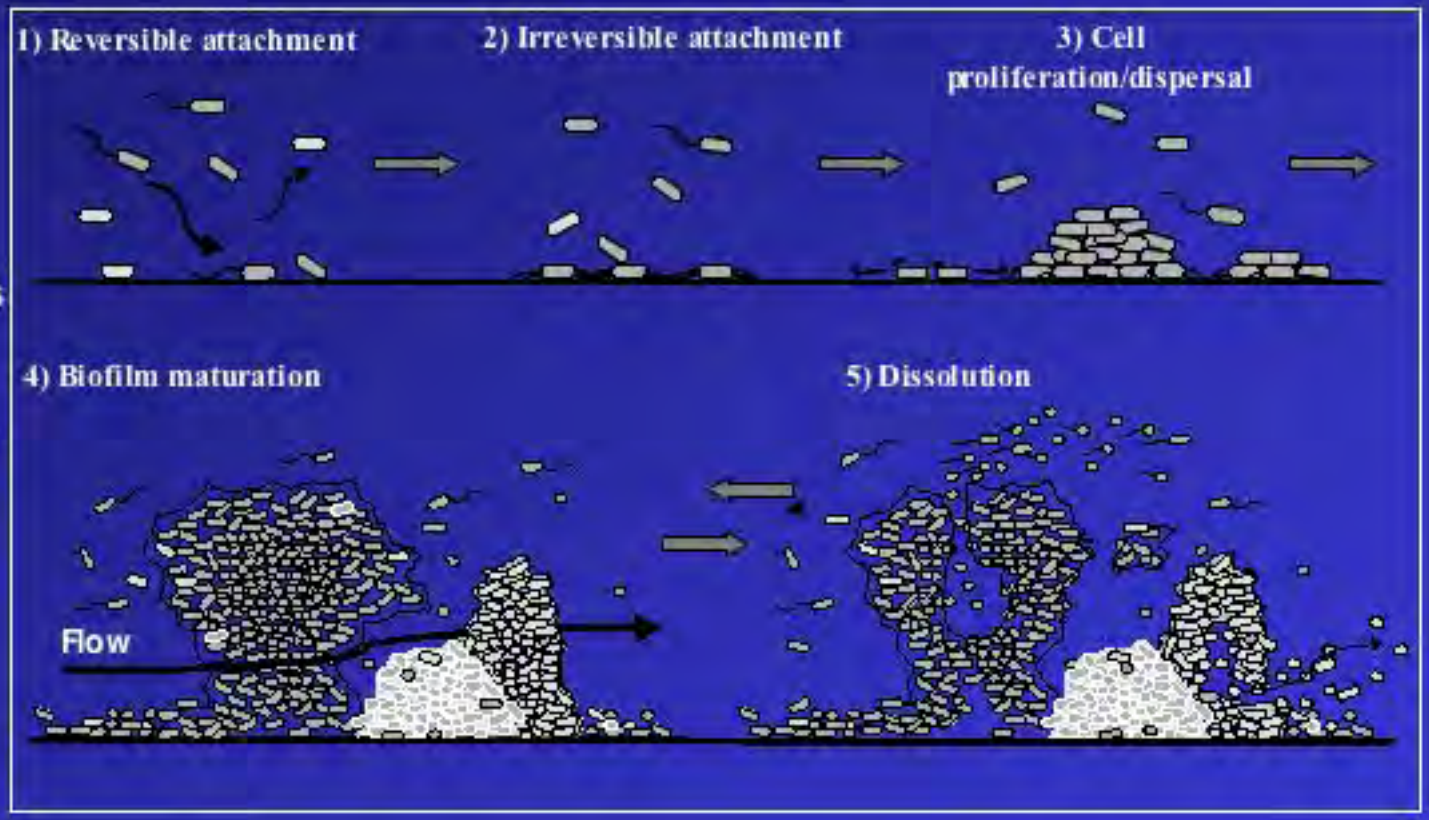
- Physiology
- Biochemistry
- Genetics
- Resistance: To innate and adaptive defence mechanisms
  To antibiotics/disinfectants
- Pathology - pathogenesis - disease

Definition: A structured consortium of bacterial cells surounded by a self-produced polymer matrix, mono/polyspecies

BIOFILM INFECTION = Chronic infection = an infection which A) persists in spite of therapy, and in spite of the host's immune- and inflammatory response, and B) is characterized by persisting pathology and immune response (in contrast to colonization)

Biofilm development – consensus?

In hydrodynamic conditions biofilm development depends on *adhesive forces*. At the substratum bacterial *motility* may have significant impacts on structure development.



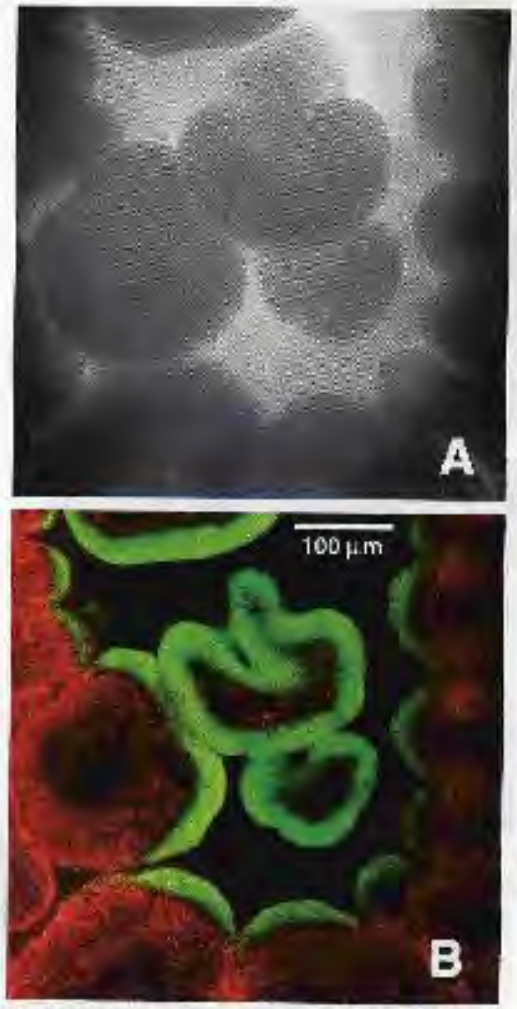


FIG. 8. Stratified pattern of GFP expression in *P. aeruginosa* biofilms grown in glass capillary tubes under continuous flow conditions. Strain PAO1(pADI; was grown for 24 h and then induced with 1 PTG for 4 h. Panel A shows a laser transmission view, and panel B shows a fluorescence image of the same spot. Green areas are due to GFP and red areas are due to the chalcamine B counterstain.

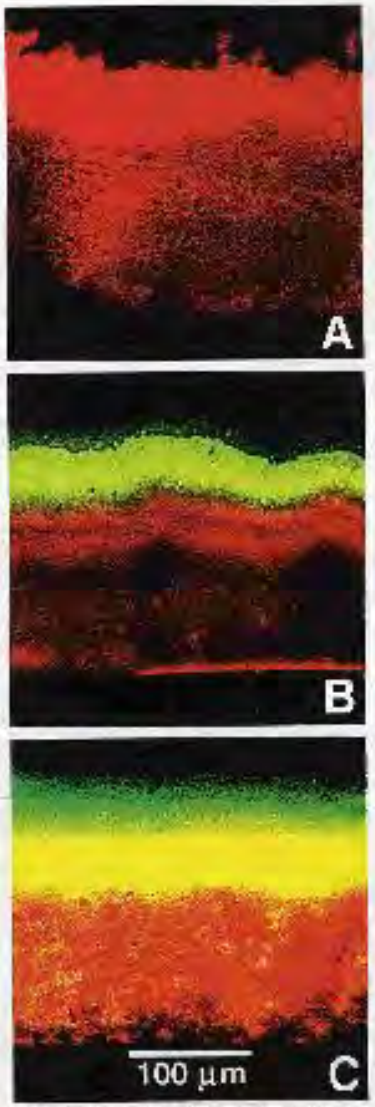


FIG. 4. Stratified patterns of GFP expression in three sections of *P. aeruginosa* colony biofilms. Green areas are due to GFP and red areas are due to the chalcamine B counterstain. Panel A shows a segment excised in which a colony biofilm formed by strain PAO1(pMF230) was not induced. Panel B shows a biofilm of the same strain after 4 h of induction with IPTG. Panel C shows a colony biofilm formed by the reporter strain MF230.

Microbiol. (Werner et al. Stratified growth in P. aeruginosa biofilms. Appl. Environment 70:6188-96; 2004)

Red: control stain of biofilm bacteria

Green = GFP = metabolic active bacteria at the biofilm surface

Some general features of biofilm infections in humans compared to acute planktonic infections and superficial colonozation/normal flora on skin and mucosal membranes. **The bold fonts indicate biofilm specific features**

Features of biofilm infections	**Necessary condition for biofilm infections**	**Sufficient condition for biofilm infecions**	**Also found in acute planktonic infections**	**Also found in colonization/ normal flora on skin and mucosal membranes**
Aggregates of bacteria embedded in a self-produced polymer matrix	Yes	Yes	No	No/Yes
Tolerant to clinical relevant PK/PD dosing of antibiotics in spite of susceptibility of planktonic cells	Yes	Yes	No	No/Yes
Tolerant to both innate and adaptive immune reponse	Yes	Yes	No	No/Yes - unknown (s-IgA)
Inflammation	Yes	No	Yes	No
Biofilm-specific antigens	No and Yes - seldom – e.g. P.seudomonas aeruginosa alginate	No and Yes - seldom – e.g. Pseudomonas aeruginosa alginate	No	No

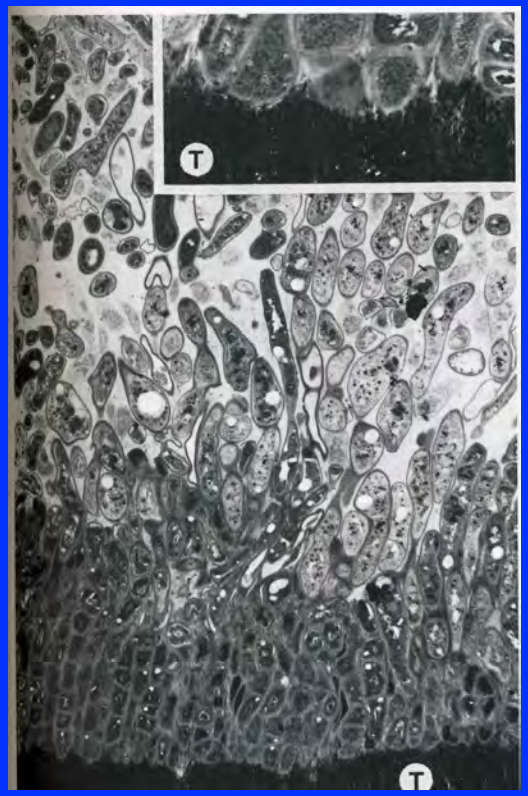
(Høiby et al.:Antibiotic resistance of bacterial biofilms Internat. J. Antimicrob. Agents 35:322-32; 2010)

Some general features of biofilm infections in humans compared to acute planktonic infections and superficial colonozation/normal flora on skin and mucosal membranes. **The bold fonts indicate biofilm specific features**

Features of biofilm infections	**Necessary condition for biofilm infections**	**Sufficient condition for biofilm infecions**	**Also found in acute planktonic infections**	**Also found in colonization/ normal flora on skin and mucosal membranes**
Antibody response	Yes - after some weeks	No	Yes - after some weeks	No
Chronic infections	Yes	Yes	No	No
Foreign body associated infections	No	Yes	No but yes the first day of infection	No
Located on surfaces	No	No	Yes	Yes
Localized infetion	Yes	No	Yes	Yes
Focus for spreading or local exacerbation	Yes	No	Yes	Yes

(Høiby et al.:Antibiotic resistance of bacterial biofilms Internat. J. Antimicrob. Agents 35:322-32; 2010)

S. epidermidis biofilm on an i.v. device. SEM



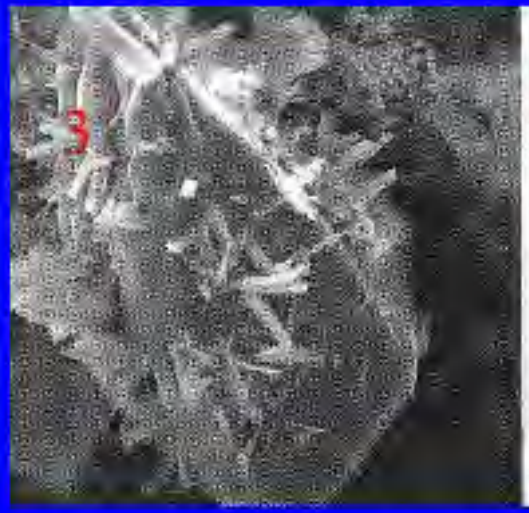
Dental biofilm, subgingival plaque (Fiehn & Larsen: Oral bacteriology. In: Høiby (ed.) Basic and Clinical Microbiologi. FADL, Copenhagen, 1998)

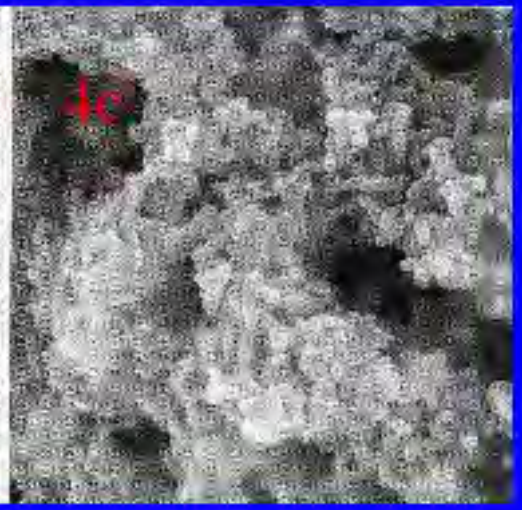
CARIES & PERIODONTITIS

DENTAL PLAQUE IS A BIOFILM. Evidence points to at least six bacterial species acting in a consortium that can lead to periodontitis, although no single species has been linked to the disease in all cases.

(Costerton & Stewart: Battling biofilms. Emerging Trends in Oral care. Scientific American Jan. 2002)

HØIBY 2011





Stickler, D.:Urinary catheters: ideal sites for the develoment of biofilm communities. Microbiology today 32:22-25; 2005

3: SEM of a biofilm on a patient's encrusted catheter. E.coli, E. faecalis & P. mirabilis were found in this biofilm community.

4 a-c: Worm-like structure that blocked a patient's catheter at 4 days interval . E faecalis, P. aeruginosa, E. coli. P.mirabilis were recovered from the biuofilm. The patient produced 15 of these worms over a 10-week period

HØIBY 2005

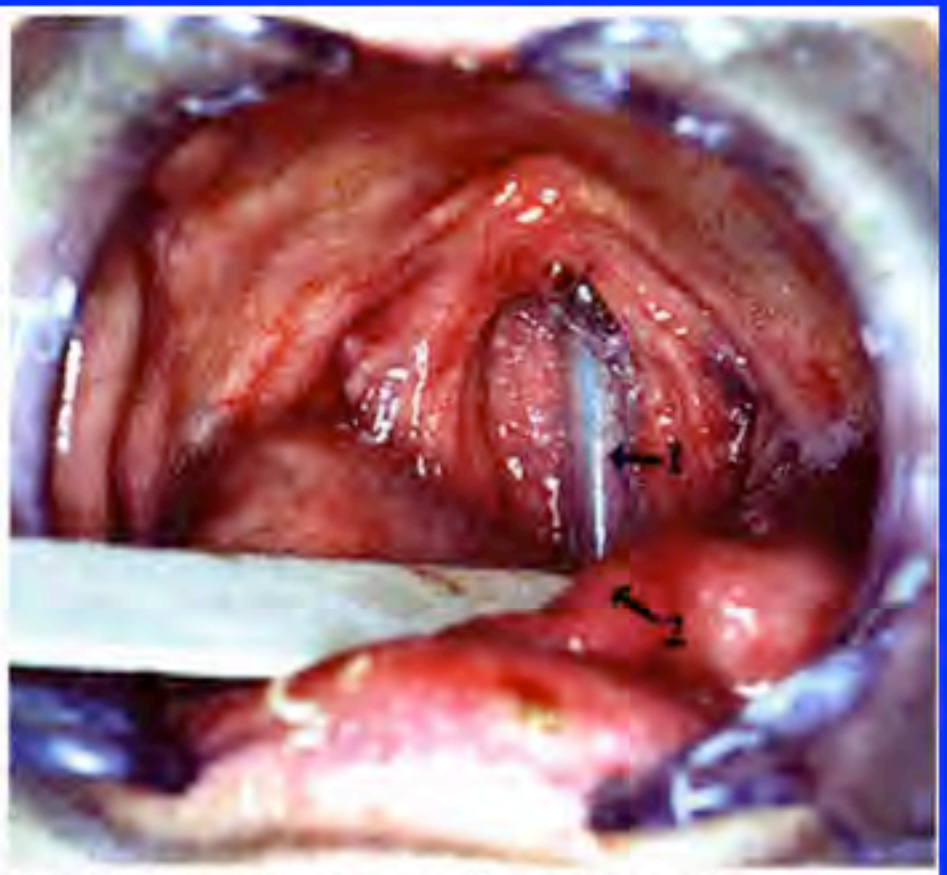


Figure 1. Nasogastric tube embedded in the nasopharynx. 1, nasogastric tube; 2, dorsum of tongue.

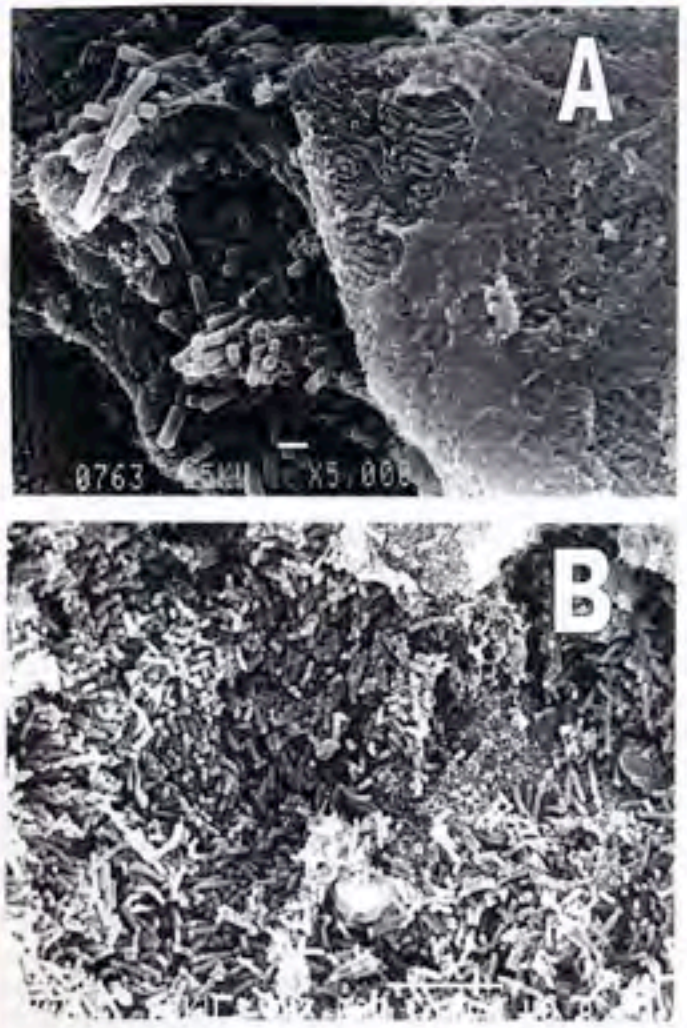
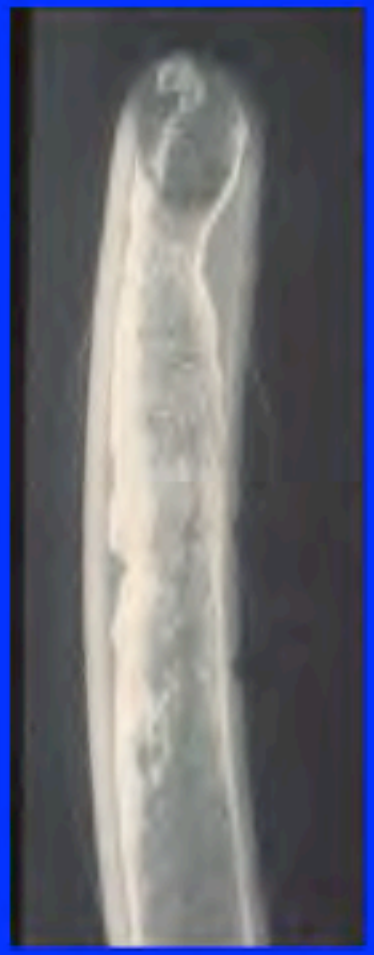


Figure 2. Representative biofilms on nasogastric tubes showing bacterial organisms with typical form of *Pseudomonas aeruginosa*. Scanning electronic microscope. A, scale bar, 1 µm; B, scale bar, 10 µm.

BIOFILM ON NASOGASTRIC TUBES (Leibovitz et al.: P. aeruginosa and the oropharyngeal ecosystem of tube-fed patients. Emerg. Infect. Dis. 9:956-59; 2003)

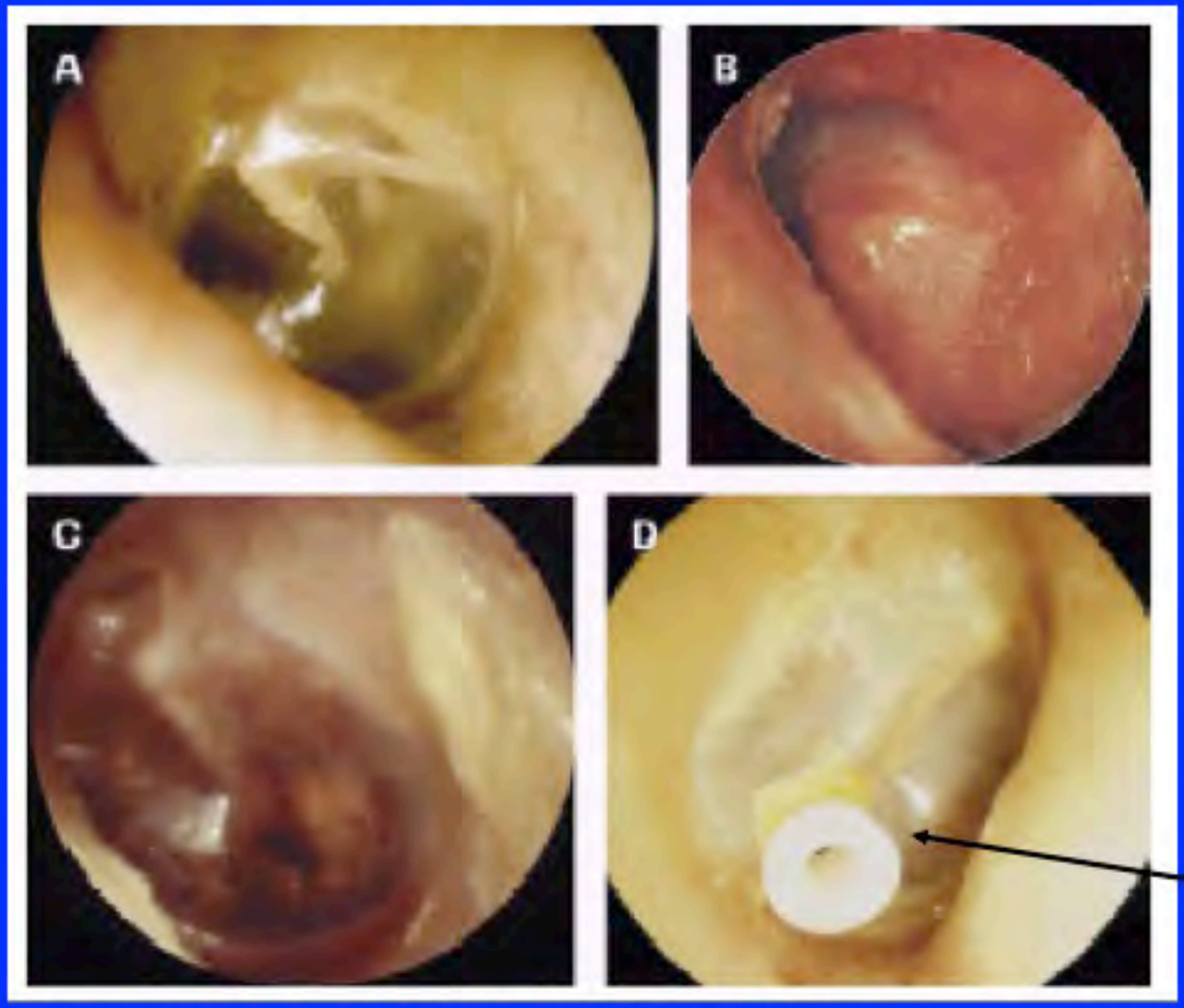


(Inglis: Evidence for Dynamic Phenomena in Residual Tracheal Tube Biofilm. Br J Anaesth1993:70:22-24)

VENTILLATOR ASSOCIATED PNEUMONIA

HØIBY 2004

BIOFILM ON VENTILLA-TION TUBES

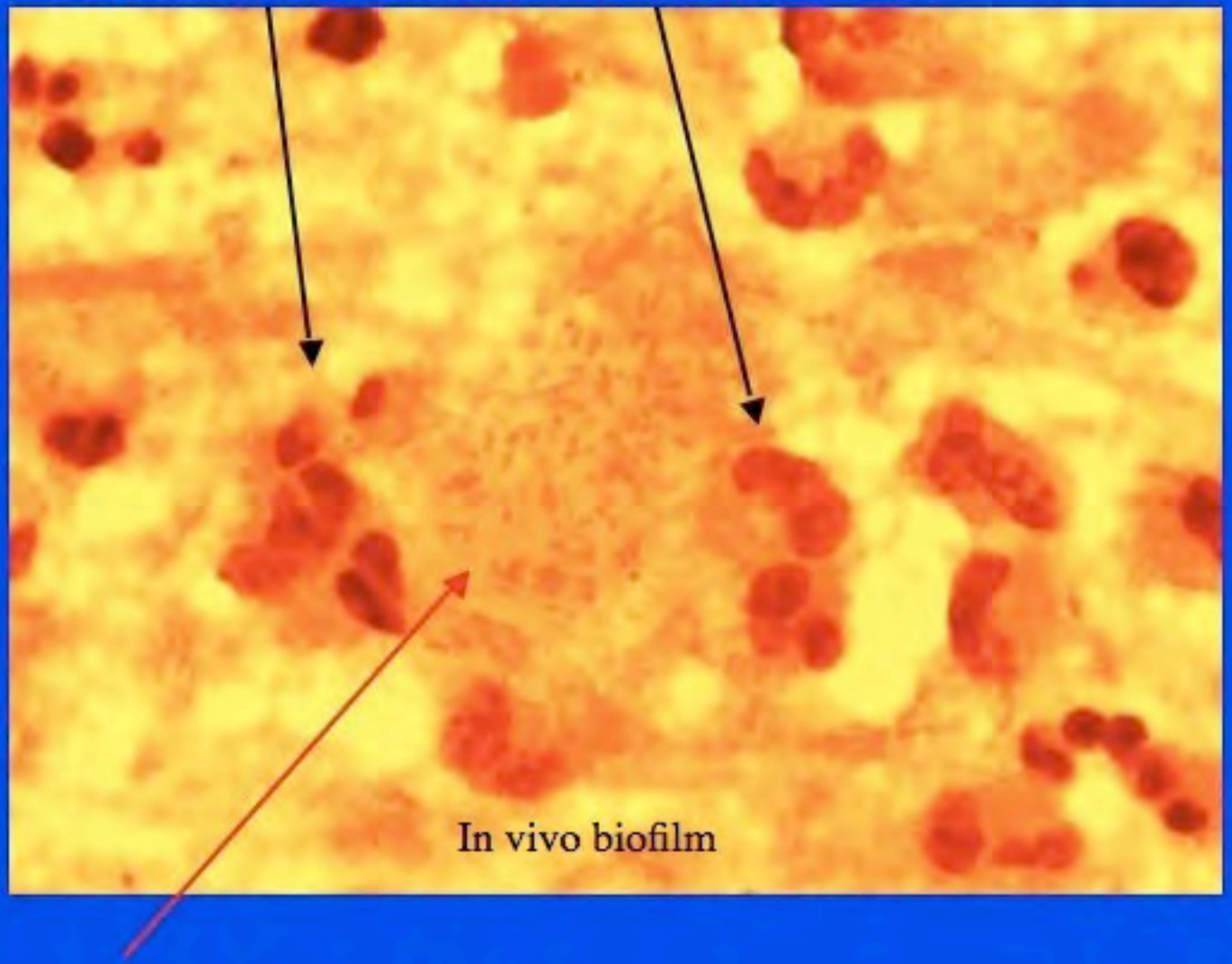


A) Normal middle ear, B) acute otitis media, C) Otitis media wwith effusion, D) Ventillation tube

(Rovers et al. Otitis Media. Lancet 363:465-73;2004)

HØIBY 2004

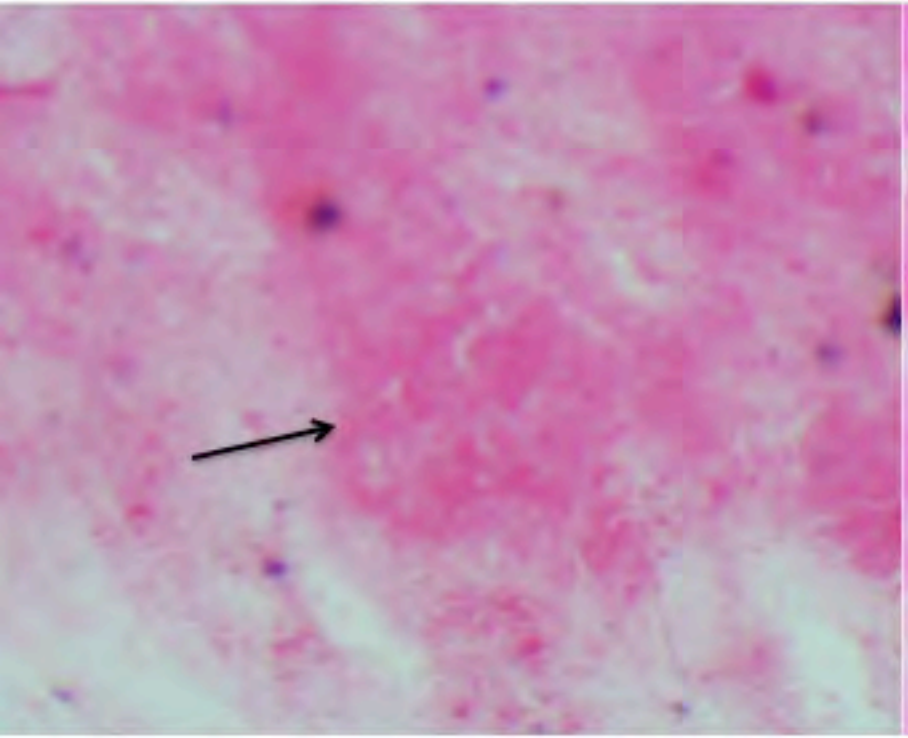
POLYMORPHONUCLEAR LEUKOCYTES



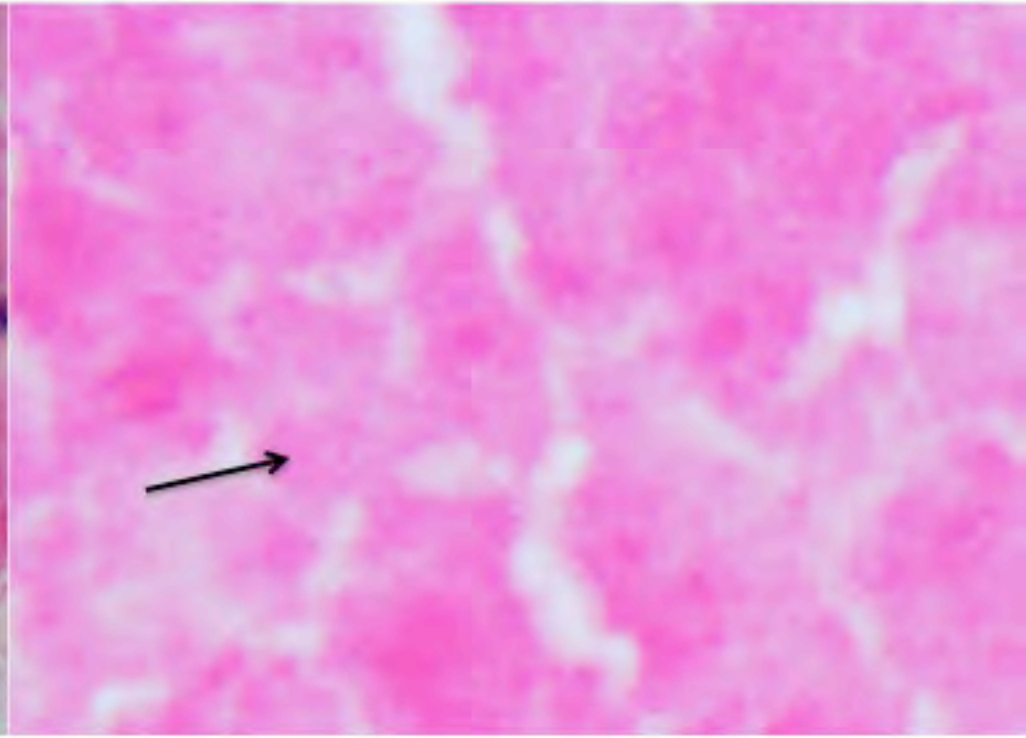
CF sputum - biofilm: **DIVERSITY!**

HØIBY 2004

Biofilm in sputum of 14 CF patients with chronic A. xylosoxidans (7/8) or B. multivorans (6/6) lung infection



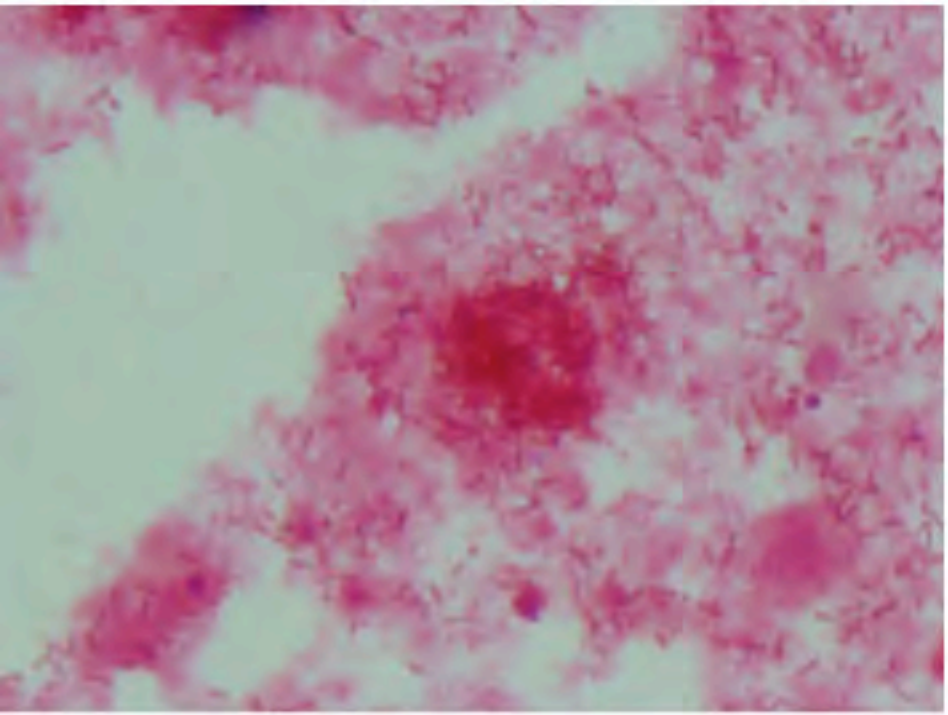
Gram stain x 40

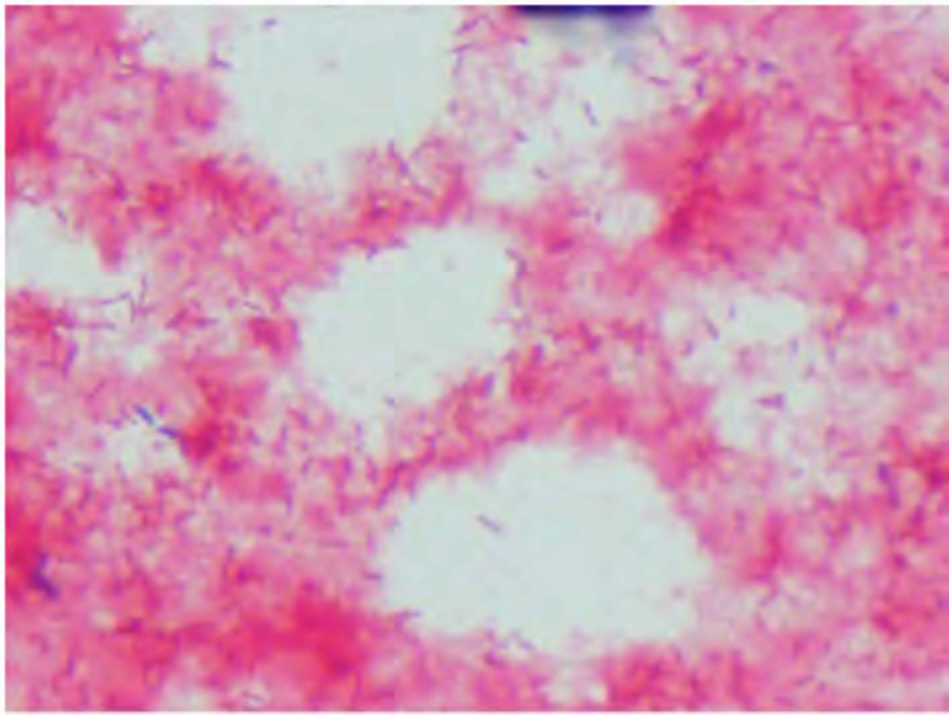


Gram stain x 1000

(Høiby 2009)

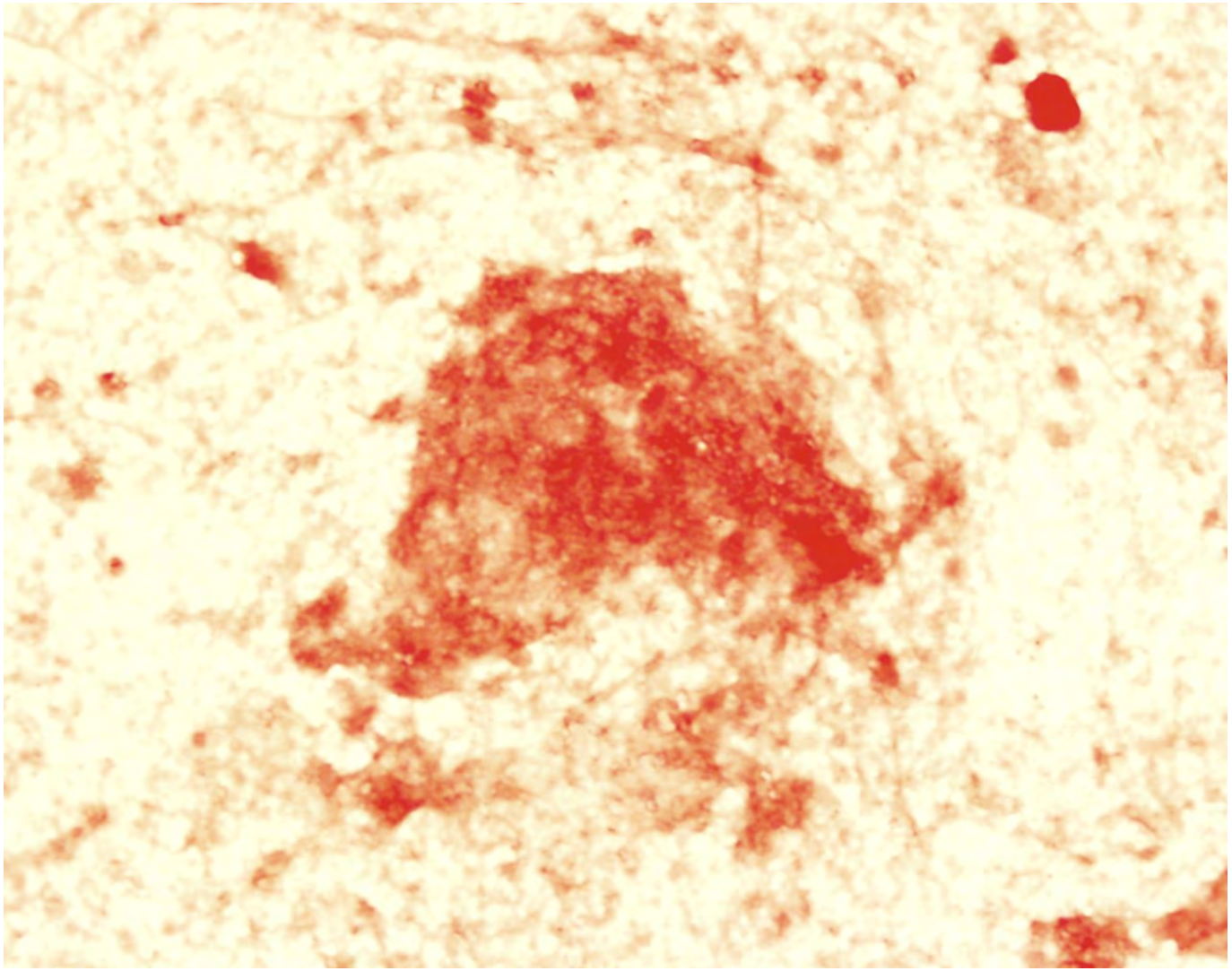
Biofilm in Sputum from two CF patients with chronic Stenotrophomonas maltophilia lung infection. Few PMNs



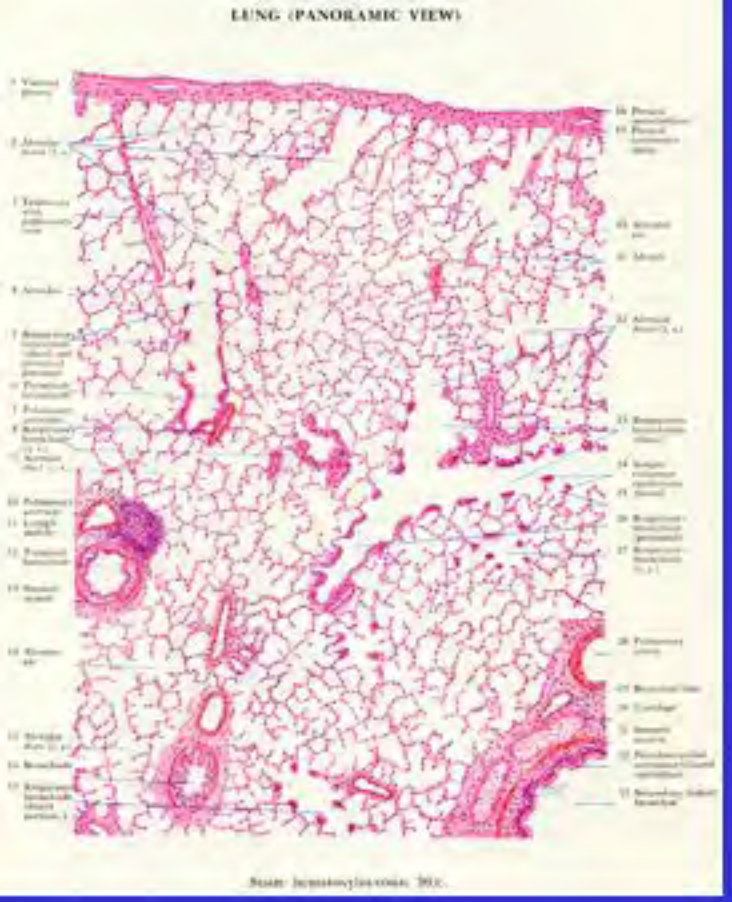


Gram stained smears x 1000

Høiby 2009

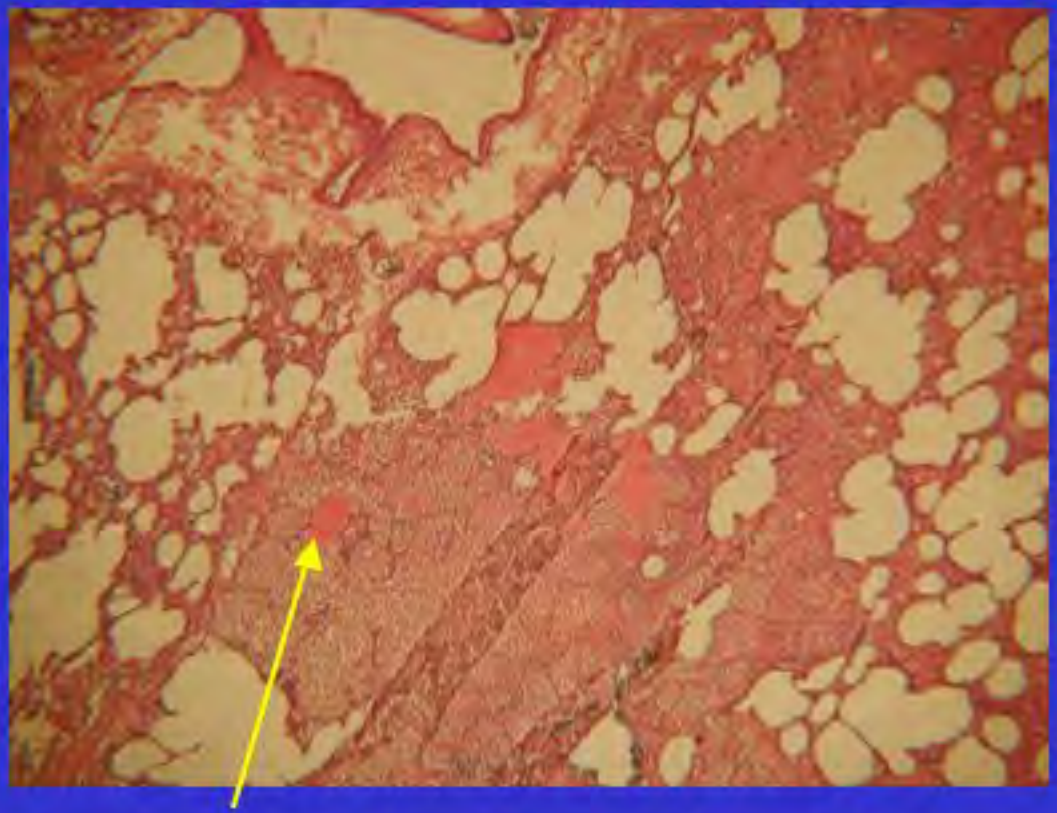


Achromobacter xylosoxidans biofilm in sputum from a chronically infected 23 year old CF patient (CF 370). Gram stain x 1000.



Normal lung

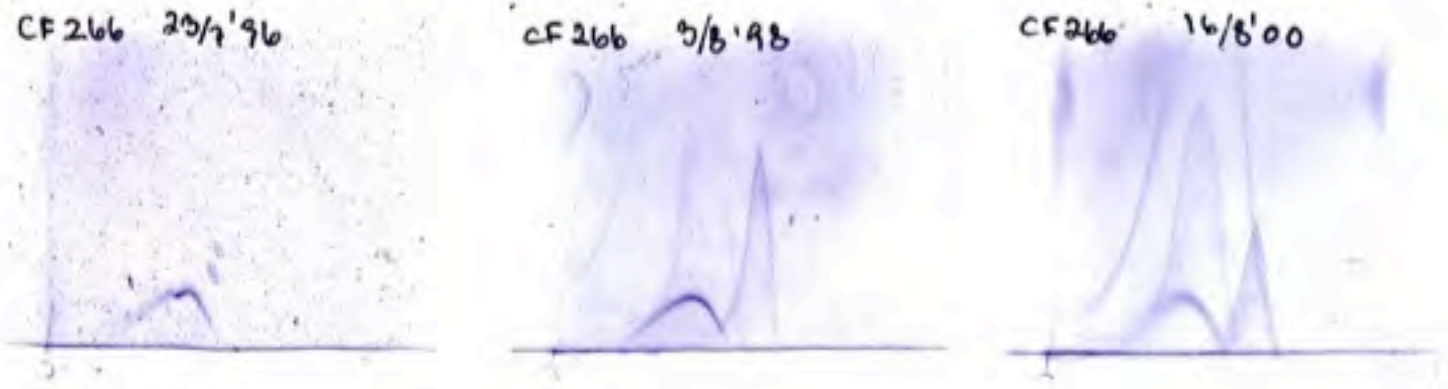
The *packed* aerobic CF alveolar incubation chamber *filled* with mucoid P. aeruginosa biofilm



Autopsy (BS242/74) of a Danish CF girl (MLM) who died due to chronic P. aeruginosa lung infection and 21 precipitating antibodies against P. aeruginosa (normal: 0-1). Severely inflammed tisssue (pneumonia). HE stain x 40.

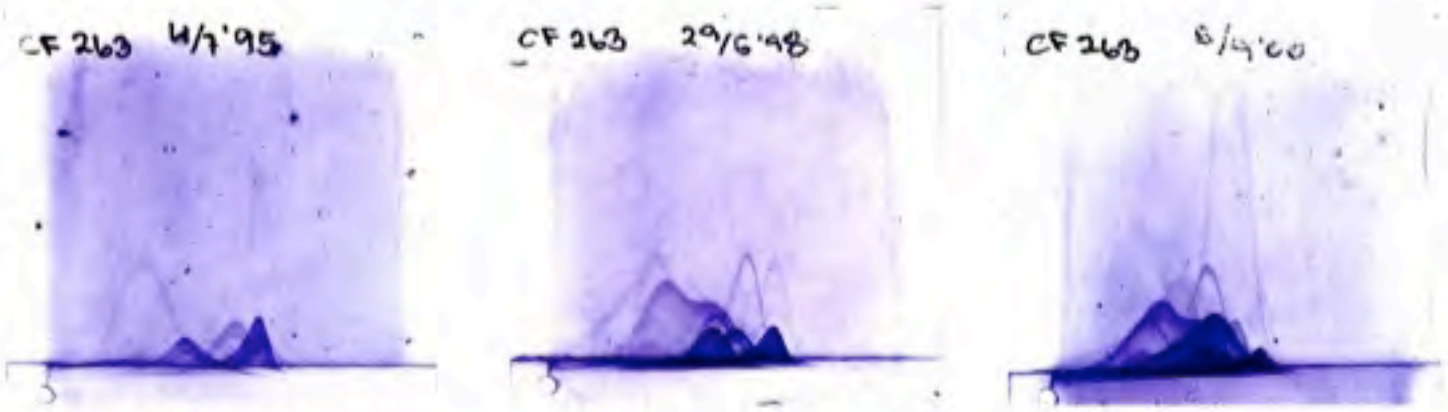
HØIBY 2004

Antibodies against P. aeruginosa measured by cossed immunelectroforesis (CIE)



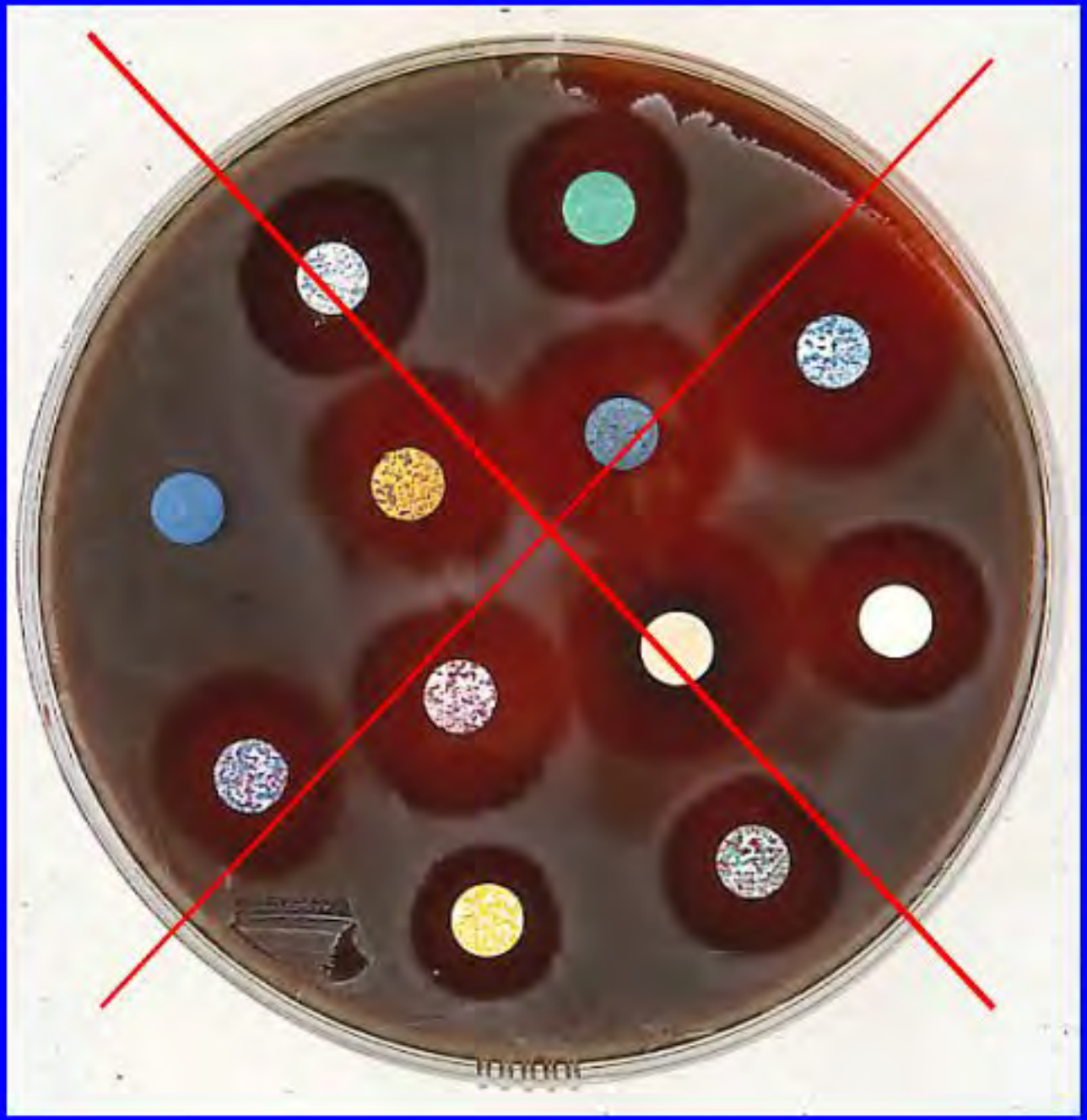
Slow increase

Antibodies – helpfull or harmfull?



Rapid increase

Normal: 0-1 precipitin



Susceptibility testing of planktonic growing bacteria does not reflect the susceptibility of the same bacteria growing as a biofilm!

Pediatric Pulmonology 46:184–192 (2011)

Randomized Trial of Biofilm Testing to Select Antibiotics for Cystic Fibrosis Airway Infection

Samuel M. Moskowitz, MD,[1,2,3]* Julia C. Emerson, MD, MPH,[2,3] Sharon McNamara, MN,[3] Richard D. Shell, MD,[4] David M. Orenstein, MD,[5] Daniel Rosenbluth, MD,[6] Marcia F. Katz, MD,[7] Richard Ahrens, MD,[8] Douglas Hornick, MD,[9] Patricia M. Joseph, MD,[10] Ronald L. Gibson, MD, PhD,[2,3] Moira L. Aitken, MD,[11] Wade W. Benton, PharmD and,[3] Jane L. Burns, MD[2,3]

Summary. Rationale: In cystic fibrosis (CF), conventional antibiotic susceptibility results correlate poorly with clinical outcomes. We hypothesized that biofilm testing would more accurately reflect the susceptibilities of bacteria infecting CF airways. Methods: A multicenter randomized pilot trial was conducted to assess the efficacy and safety of using biofilm susceptibility testing of *Pseudomonas aeruginosa* sputum isolates to guide antibiotic regimens for chronic airway infections in clinically stable adolescent and adult CF patients. Thirty-nine participants were randomized to biofilm or conventional treatment groups; 14-day courses of two antibiotics were selected according to an activity-based algorithm using the corresponding susceptibility results. Results: Of the agents tested, meropenem was most active against biofilm-grown bacteria, and was included in regimens for about half of each study group. For 19 of 39 randomized participants, randomization to the other study group would not have changed the antibiotic classes of the assigned regimen. Study groups were comparable at baseline, and had similar mean decreases in bacterial density, measured in $\log_{10}$ colony forming units per gram of sputum (biofilm, −2.94 [SD 2.83] vs. conventional, −3.27 [SD 3.09]), and mean increases in forced expiratory volume in 1 sec, measured in liters (0.18 [SD 0.20] vs. 0.12 [SD 0.22]). Conclusions: In this pilot study, antibiotic regimens based on biofilm testing did not differ significantly from regimens based on conventional testing in terms of microbiological and clinical responses. The predictive value of biofilm testing may nonetheless warrant evaluation in an adequately powered clinical trial in younger CF patients or those experiencing acute pulmonary exacerbation. **Pediatr Pulmonol. 2011; 46:184–192.**

Key words: ***Pseudomonas aeruginosa*; intravenous antibiotics; antibiotic resistance; antibiotic susceptibility testing; broth microdilution testing; inhibitory quotient; sputum bacterial density; lung function.**

Comparison of biofilm and planktonic susceptibility testing of CF *P. aeruginosa* isolates

Antibiotic 90 strains	MIC_{50} (μg/ml) (range)	BIC_{50} (μg/ml) (range)
Piperacillin/Tz	4 (<1-1024)	256 >512
Ceftazidime	2 (<0.5-512)	128 (<2->128)
Aztreonam	4 (<2-64)	>128 (<2->128)
Meropenem	< 1 (<1-16)	4 (<1->64)

(Moskowitz, S., J. Clin Microbiol. 2004, 42:1915-22)

Oana Ciofu 2005

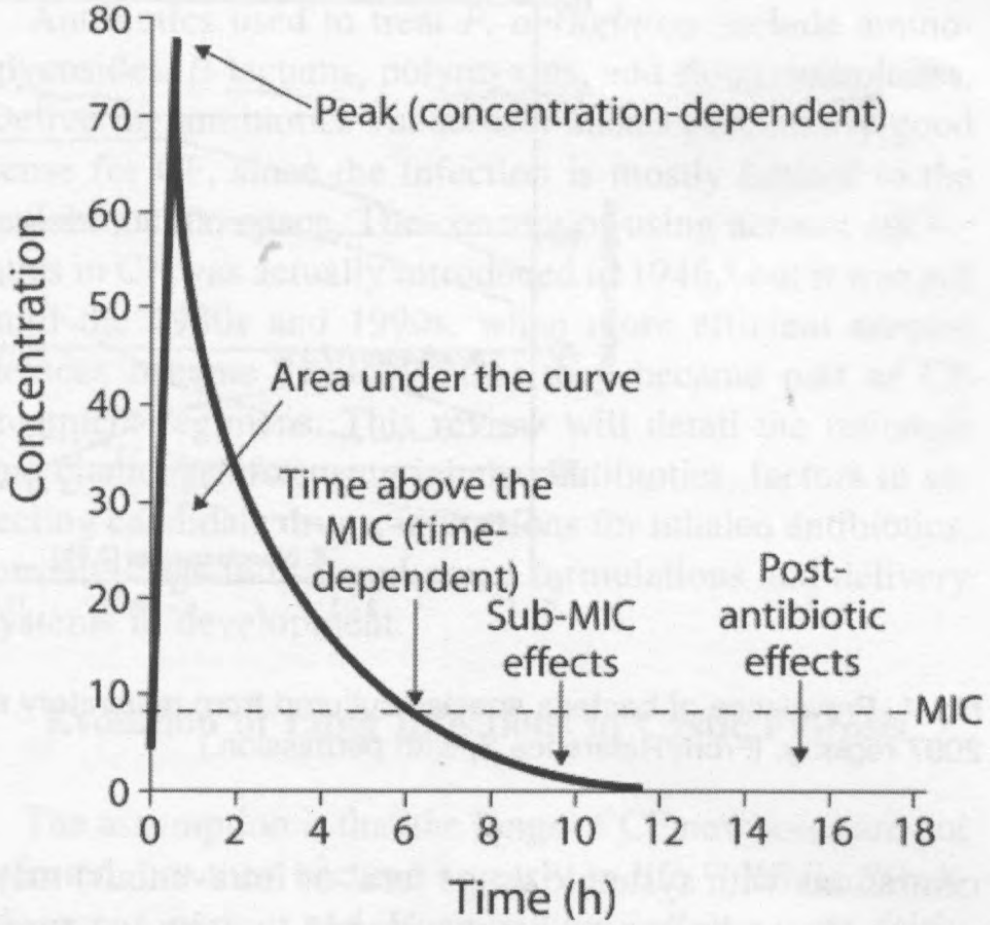


Fig. 3. Antibiotic pharmacodynamics. The graph is a representative concentration-versus-time curve of an antibiotic in the compartment of interest (in this case, the lungs). After inhalation, the level is very high, then falls due to absorption into the bloodstream or elimination through airway clearance. β-lactam antibiotics demonstrate time-dependent killing (the longer the time above the minimum inhibitory concentration [MIC] of the bacteria, the better). Aminoglycosides and fluoroquinolones demonstrate concentration-dependent killing (a high ratio of average maximum serum concentration to MIC or area-under-the-curve [AUC] to MIC work best).

Summary

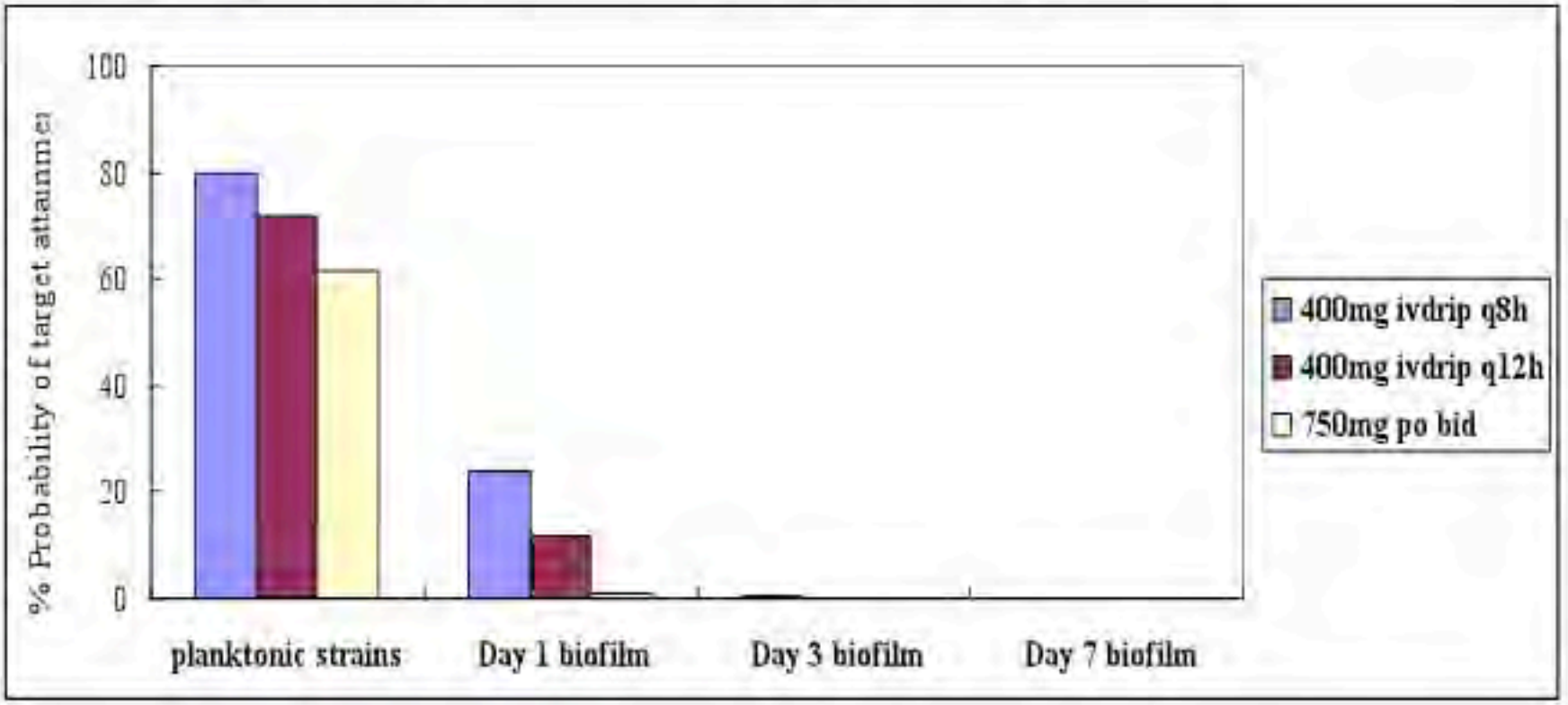
Minimal bactericidal concentration (MBC)
minimal biofilm eradication concentration (MBEC)
Minimal biofilm inhibition concentration (MBIC)

	MBC (planktonic) & MBEC (biofilm) (μg/ml)	
	Ciprofloxacin	**Imipenem**
Planktonic	**2**	**8**
Day 1 biofilm (non-mucoid PAO1)	**8**	**256**
Day 1 biofilm (Mucoid PDO300)	**32**	**>256**
Day 3 biofilm	**32**	**>2048**
Alginate beads	**64**	**>256**
PK/PD index	***f*C_{max}/MIC (planktonic)** ***f*AUC/MIC (planktonic)**	***f*T>MIC (planktonic)**
	***f*C_{max}/MBIC (biofilm)** ***f*AUC/MIC (biofilm)**	***f*T>MBIC (biofilm)**

(Wang, Song, Wu & Høiby 2010)

Ciprofloxacin Population Pharmacokinetics / Monte Carlo Simulation on CF patients on the basis of published data

※Target: *f*AUC0-24/MIC ratio ≥ 125 (mg · h / liter)



(Alan Forrest, AAC, 1993; Pedersen S. S, JAC, 1987; Montgomery M. J, AAC, 2001)

(Wang, Song, Wu & Høiby 2010)

Biofilm Infections - Prevention & Therapy

- Prophylaxis: 1) antibiotic prophylaxis, 2) Early aggressive antibiotic therapy when infection is suspected hopefully before the biofilm is fully mature, 3) Antibiotic- coated or silver-coated or antiseptic-solution-coated foreign body
- Therapy: Biofilm infections cannot be eradicated by antibiotic therapy. The strategy is therefore either to replace the foreign body (if possible) covered by antibiotic therapy, and preferably after ≥24 h interval to insert a new, clean foreign body using "prolonged" pathogen-directed antibiotic prophylaxis for 14 days (until endothelial/epithelial growth and coverage of the surface/insite place is finished. If that cannot be done, then the strategy is to use continuous suppression of the biofilm growth by giving the patient pathogen directed antibiotic treatment for prolonged time, in some cases (e.g. aortic grafts) for the rest of the life of the patient

I WILL BE HAPPY TO ANSWER ANY QUESTION.....

.....FROM THE AUDIENCE.....

Høiby 2009